Critical role of the blood coagulation in Malaria: *Plasmodium falciparum*-infected red blood cells induce Tissue Factor expression by Endothelial Cells and support the assembly of coagulation complexes

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Malaria caused by *Plasmodium falciparum* and transmitted by the mosquito vector *Anopheles gambiae* is still the world's most important parasitic disease with 300-500 million people infected every year. Deaths occur mostly among young children in Africa. Cerebral malaria (CM) is the most severe complication and accounts for up to 80% of fatal cases. The mechanisms of malaria and CM pathogenesis remain the subject of debate. It is believed that hemostatic alterations could be of importance in the disease since *P. falciparum* malaria has been associated with thrombocytopenia and hemostatic alterations (pro-coagulant state). Here we demonstrate that *P. falciparum*-infected red blood cells (IRBC) induce Tissue Factor (TF) expression by Endothelial Cells (EC) in culture, in a parasitemia- and time-dependent manner. TF was consistently detected by functional (Factor Xa formation), antigen (ELISA), RT-PCR, Western-blot and immuno-histochemistry assays. Remarkably, mid-late but not early trophozoites and ring stage parasites were capable of inducing TF expression. These findings are compatible with *post-mortem* brain biopsies where sequestration is composed mostly by late-stage trophozoites. Our experiments also demonstrate that late-stage IRBC support the assembly of the prothrombinase complex (FVa, FXa, prothrombin and Ca^{2+}) with thrombin formation. IRBC also support the intrinsic Xnase complex assembly (FIXa, FVIIIa, FX and Ca^{2+}) with FXa production. It is concluded that IRBC manipulates the hemostatic system by initiating the blood coagulation cascade through induction of TF expression by EC, with formation of FVIIa/TF complex (extrinsic Xnase) and FXa generation. FXa participates in the prothrombinase assembly in the IRBC surface, allowing the coagulation cascade to propagate, with thrombin formation. Thrombin, FXa and FVIIa may also activate the pro-inflammatory PAR receptors in EC creating a unique inflammatory environment in the vessels where sequestration has occurred. These findings introduce the concept that malaria is an inflammatory syndrome triggered by expression of TF with activation of the coagulation cascade. Identification of TF as a critical mediator of this disease may allow investigators to test other therapeutic alternatives targeting TF in the treatment of malaria and its complications.

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